Chemosphere 74 (2008) 142–148

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Ecorisk evaluation and treatability potential of soils contaminated with petroleum hydrocarbon-based fuels

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ARTICLE INFO

*Article history:*
Received 15 May 2008
Received in revised form 7 August 2008
Accepted 13 August 2008
Available online 27 September 2008

*Keywords:*
Microtox
Bioremediation
Hydrocarbon
Bioassay
Oil lakes
Organic pollutants

ABSTRACT

We used a series of toxicity tests to monitor oil degradation in the Kuwaiti oil lakes. Three soils from different locations with a history of hydrocarbon contamination were treated in bench-scale microcosms with controlled nutrient amendments, moisture content, and temperature that had promoted mineralization of total hydrocarbon and oil and grease in a preliminary study.

Two hundred days of bioremediation treatment lowered hydrocarbon concentration to below 2 and 5 mg $g^{-1}$ for soils A and B, respectively, while in soil C hydrocarbon concentration remained at 12 mg $g^{-1}$. Although 85% of the total petroleum hydrocarbons (TPHs) in soil A were reduced 50 d after treatment, results of the seed germination and Microtox tests suggested an initial increase in toxicity, indicating that toxic intermediary metabolites may have formed during biodegradation. Also, the significant decrease of TPHs and corresponding high toxicity levels were noted in soil B 200 d after bioremediation.

Clearly, toxicity values, and not just hydrocarbon concentration, are a key factor in assessing the effectiveness of bioremediation techniques. Field chemistry data showed a significant reduction in hydrocarbon levels after the biological treatment. We concluded that the toxicity assessment of the contaminated soil with a battery of toxicity bioassays could provide meaningful information regarding a characterization procedure in ecological risk assessment.



## 1. Introduction and background

Oil is a highly complex assemblage of organic compounds that no single analytical method can entirely characterize. Tracing the fate of spilled oil requires monitoring bulk oil concentration changes along with detailed composition changes in the oil itself. Several tests have been proposed as indicators of soil quality in evaluating damage and risk to affected ecosystems (Coover and Sims, 1987; Graham et al., 1995; Margesin and Schinner, 1997).

Hydrocarbons, measured mainly as total petroleum hydrocarbons (TPHs), contain the bulk of components in nearly all crude oils and include a combination of chemical components. Several of these components are toxic and included in the list of US Environmental Protection Agency (EPA) priority pollutants (Bojes and Pope, 2007). Soil and groundwater pollution with petroleum hydrocarbon-based fuels as a result of accidental spills or inappropriate storage has been described previously (Herbes, 1981; Erickson et al., 1993; Li et al, 1995; Canet et al., 2001; Namkoong et al., 2002; Antizar-Ladislao et al., 2004; Cai et al., 2007). These chemicals pose serious health threats and ecological stresses as a result of the discharge of volatile hydrocarbons into the environment and from the unintentional infiltration of oily materials into soil and groundwater.

Several physiochemical techniques have been evaluated for the treatment of soil contaminated with hazardous material such as petroleum hydrocarbons. These techniques include vapor extraction, stabilization, solidification, soil flushing, soil washing, thermal desorption, vitrification, and incineration (Zappi et al., 1996; Balba et al., 1998). Yet most of these techniques require expensive continuous monitoring, often limiting the frequency and number of stage evaluations. Bioremediation, for instance, can be an efficient and inexpensive choice compared to physiochemical methods. This method provides a microbial population (plants, fungi, and bacteria) that can degrade the available organics found in the contaminated soil. Also, reduced contaminant concentrations do not always indicate decreased soil toxicity (Baud-Grasset et al., 1992; Loehr and Webster, 1996). Moreover, incomplete degradation and formation of toxic intermediary metabolites or transformation by-products may result in increased soil toxicity during bioremediation (Loehr and Webster, 1996; Loibner et al., 2003).

Also, to assess oil-contaminated soil, hydrocarbon disappearance alone may mean that the oil simply washed away or was not bioavailable. Therefore, a combination of chemical analysis and toxicity testing is recommended to account for these effects

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doi:10.1016/j.chemosphere.2008.08.020

and to help evaluate the risk associated with this type of contamination. Thus, extensive monitoring and a final evaluation of remediation efficiency are crucial for bioremediation process control as well as for environmental safety considerations.

Monitoring is usually a mandatory element of any new response or spill alleviation technique to ensure a positive environmental advantage. Monitoring must provide a quantitative assessment to evaluate efficacy or proof of action. At present, there are no common cleanup standards that have been adopted for soils contaminated with crude oils. Most monitoring studies rely on the results of chemical analyses such as gas chromatography–mass spectrometry (GC–MS) to evaluate efficacy and infer residual oil toxicity (Barthe, 1986).

The oil spill in Kuwait that led to the formation of more than 300 oil lakes covering an area of about 50 $km^2$ provided a "spill of opportunity" for evaluating the efficiency of the soil cleanup assay. Seventy oil lakes were formed in shallow depressions and distributed in the southern and northern oil fields. The volume of oil contained in the oil lakes is estimated at nine million $m^3$, with oil penetration into the underlying soil of more than 2.5 m (Al-Awadhi et al., 1993).

The solid-phase Microtox test (Benton et al., 1995; Ringwood et al., 1997) and the seed germination assays (Trevors, 1999) were used to measure soil toxicity. The Microtox assay has been used to gauge the reaction of the luminescent bacterium *Photobacterium phosphoreum* to chemical agents such as hydrocarbons in sediments (Dorn et al., 1998; Marwood et al., 1998). The procedure gives a direct measure of toxic response rather than quantitative values, which merely infer toxicity. The endpoint measured by Microtox is a reduction in the light intensity produced by the luminescent bacteria. The concentration of toxicant required to reduce the light intensity to 50% is called the $EC_{50}$ value. This strain of bioluminescent bacteria is tremendously sensitive to organic toxicants and mixtures of toxicants. Owing to the complex matrix obviously found in crude oil treatment by-products, this test can assess the toxicity of the remaining oil and not simply individual components in the oily residue.

The success and specificity of luminescent bacteria in determining toxic concentrations of organics and heavy-metal pollutants has been documented previously (Kwan and Dutka, 2004). Information from Microtox screening has also been used to evaluate sediment bound to organic contamination (Athey et al., 1989). EPA reported the method as a toxicity assay procedure for use among soil and soil–waste mixtures to set up concentration criteria for oil and sludge bioremediation (Greene et al., 1988). Furthermore, the Microtox test has been evaluated against other biological species toxicity assays such as rainbow trout and flathead minnow, with satisfactory correlations and at significant savings in time and cost (Marwood et al., 1998).

On the other hand, different toxicity experiments are anticipated to respond differently to individual toxicants. Furthermore, physiochemical and biological parameters of soil also affect toxicity to a particular test organism. A series of toxicity tests will definitely be useful to provide an overall assessment of the progress of bioremediation in contaminated soils (Marwood et al., 1998). Also, seed germination and plant growth have been used for evaluating treatment endpoints and restoration of oily contaminated sites (Amakiri and Onofeghara, 1984).

This study considered the applicability of a direct toxicity measuring system to evaluate changes in apparent toxicity and used the quantitative values to assess bioremediation efficacy. The Microtox system and seed germination were evaluated as a possible monitoring device to partly replace expensive GC–MS analyses in a comprehensive environmental monitoring system. This report had the following objectives: (1) Determine the limits and extent of hydrocarbon biodegradation, plant toxicity, and waste leachability of oil-containing soils. (2) Synthesize the experimental toxicity data in conjunction with residual oil chemistry data collected during the complementary chemical monitoring study.

## 2. Materials and methods

### 2.1. Soil preparation and analysis

We collected samples from three soils (one from the northern part [soil A] and the other two from the southern part [soils B and C] of Kuwait) with a history of oil pollution. Each soil was passed through a 4.75-mm-sized screen, mixed completely, and stored in preserved containers at 4 °C in the dark. The moisture content and water-holding capacity were determined for each soil by the method of Atlas and Bartha (1992). Soil texture, pH, and nutrient levels were determined by the Environmental Engineering Laboratory, Kuwait University. Three control samples were also collected: (1) uncontaminated agricultural control soil (soil D) collected from a potato-growing field in the southern part of Kuwait, (2) uncontaminated clean soil (soil E) from the northern part of Kuwait, and (3) untreated oil-contaminated soil (soil F) from the northern part of Kuwait.

Moreover, microcosms were prepared in 4-L glass jars containing the equivalent of 1.5 kg (dry weight) of soil. The microcosms were sealed and incubated at 20 °C in the dark and were aerated by removing the lids for 10 min, twice weekly. Each microcosm was weighed biweekly, and deionized water was added, if necessary, to replace lost moisture. Sampling was done on day 0, 50, 100, 150, and 200 for all tested soils. TPH analysis was measured using Method 9070 in EPA SW-846 with an additional extraction step. TPH analysis did not include nonpetroleum fractions, such as animal fats and humic and fulvic acids. Replicate samples (50 g wet weight) from each treatment were taken to determine TPH values.

### 2.2. Substrate functional analysis

We used the Biolog system, which is based on the inoculation of 96-well microtiter plates with bacteria from the soil samples. Every well holds a tetrazolium dye and a carbon source that is distinctive to the well and acts as a basal nutrient medium, which may or may not sustain microbial growth. Twenty-gram (dry weight) samples were brought up to 65 mL to a 10 mM phosphate buffer (pH 7.4) and then shaken (320 rpm) on an orbital shaker (20 min). Samples were allowed to settle for 10 min and Biolog GN (Gram-negative) microtiter plates were directly inoculated with 100-mL aliquots of the samples according to the methods of Guckert et al. (1996).

Plates were incubated at 35 °C until they produced an average well color development (AWCD) of approximately 0.75 absorbance units (Konopka et al., 1998). Color formation in the individual cells of the microtiter plates was measured with an Anthos reader 2001 (Anthos Labtec Instruments, Frederick, Maryland, USA) at a wavelength of 590 nm.

### 2.3. Soil toxicity analysis

The solid-phase Microtox test used *P. phosphoreum* to monitor acute toxicity of soil samples (Benton et al., 1995; Ringwood et al., 1997). $EC_{50}$ data were determined using Microtox Data Capture and Reporting software (Microbics Corp., Carlsbad, CA). The samples were solvent extracted by dichloromethane after standard extraction methods for trace analysis. The sample size was 50 g of wet sediment. Each extract was filtered through an alumina/silica gel column to remove highly polar biogenic compounds and trea-

ted with activated copper to remove inorganic sulfur. Each sample was assayed on a Microbic Model 500 Analyzer. Bioluminescence was measured after a 20-min exposure period at 15 ± 1 °C. The effect was expressed as the sediment concentration that caused a 50% reduction in bioluminescence ($EC_{50}$; expressed in milligrams per liter of sediment).

We also performed the lettuce seed germination assay in triplicate by using *Lactuca sativa*. One hundred grams of soil were placed in 150-mm disposable Petri dishes. Forty lettuce seeds were pressed into the soil in each dish. Test soils were hydrated to 85% of water-holding capacity with deionized water. A cover of 90 g of artificial soil was poured on top of the hydrated test soil. Petri dishes were placed without their cover into a polyethylene bag. Bags were sealed, leaving as much space as possible to provide sufficient air for plant seed oxygenation, and incubated at 20 °C in the dark for 48 h followed alternately by 16 h of light and 8 h of dark for the next 72 h. After 5 d of incubation, replicates were examined for the total number of emerged lettuce seedlings (at or above the soil surface), germinated seeds (beneath the soil surface), and the root elongation tests as described by Greene et al. (1988). Probit analysis was used to determine the 50% lethal concentration ($LC_{50}$) and $EC_{50}$ values for germination and emergence, respectively (Finney, 1971). The $LC_{50}$, calculated in the seed germination test, is the concentration of soil that is estimated to be lethal to 50% of the seed plants within the test period. The $EC_{50}$, calculated in the root elongation test, is the concentration that reduces the average root elongation of the plant seeds by 50% within the test period. Statistical differences among subsamples were determined using a one-way analysis of variance. Statistical significance was evaluated for $P$ values less than 0.05.

### 2.4. Soil leaching potential

We assessed the leaching properties of the soil samples by following the US EPA Toxicity Characterization Leaching Procedure (TCLP) (US EPA Method 1311, 1990). Twenty grams of the solid samples were placed in polyethylene bottles followed by the addition of 800 mL of acetic acid solution (5.7 mL of acetic acid in 1 L of distilled water). The bottles were placed in a cubical rotator and were rotated for 1672 h at 32 rpm. After rotation, samples were filtered and the filtrates were assessed for their metal content. Inductively coupled plasma–atomic emission spectrometry was used to analyze metals in the TCLP extract. The toxic properties of the filtrates were also assessed by *Vibrio fischeri* bioassays. GC–MS was used to analyze benzene, toluene, ethylbenzene, and xylene. The standards were prepared as specified in US EPA Method 8020B (US EPA Method 8020B, 1992).

### 2.5. Statistical analysis

The results are reported as mean from individual determinations with at least three replicates. Statistical differences were analyzed using the analysis of variance (ANOVA) as a statistical technique. The test was performed at confidence intervals of 95% and values of $P < 0.05$ were regarded as significant. A computer program (Microcal origin) was used for this purpose.

## 3. Results and discussion

### 3.1. Chemical and soil analysis

Soil pH, texture, organic carbon, and mineral contents are shown in Table 1. The northern soil samples (soils A, E, and F), sandy loam and sandy clay loam, contained the highest total phosphorus at 10 ppm. The southern soil samples (soils B, C, and D), sandy loam and loamy sand, contained the highest total nitrogen at 5.0 ppm. Soils A and B had organic carbon concentrations of 5.3% and 7.0%, respectively, two to three times higher than in soil C (2.5% each). Soil pH values (6.5–8.1) were within a reasonable range for microbial activity in all three soils.

### 3.2. Substrate functional analysis

Repeated temporal monitoring of the color of the Biolog wells and calculation of the AWCD were recommended by Garland and Mills (1991). To counterbalance the unequal cell densities in the samples, we prolonged incubation periods and transformed the data by division by the AWCD of each sample (Garland and Mills, 1991). Flat or depressed AWCD would signify little or no microbial involvement, whereas strong positive responses would indicate a large biodegradative contribution.

There were strong positive changes in number of active wells and AWCD during bioremediation at intervals of 0, 50, and 200 d. In the undegraded state at time 0, all soil values of AWCD were in the range of 0.25–0.39. They were not significantly different from each other ($P > 0.7$). However, after 50 d of bioremediation, large values of AWCD (1.25–1.30) were detected in soils A and B. The process resulted in a four- to fivefold increase in AWCD in soils A and B compared with a onefold increase in soil C. The values in AWCD were three- to fourfold lower in heavily contaminated soil C than that in soil with a lower initial contamination (soils A and B). High concentrations of toxic oil components initially inhibited soil microbial activity. However, as oil bioremediation proceeded, the values of AWCD changed, and at the final stage of bioremediation, the AWCD values of soils A and B were nearly twofold higher than that of soil C. The number of active wells and AWCD values were relatively high for aerobic microbial activity of all soil samples. However, these values decreased to their background level after the peak of the maximum oil biodegradation at 50 d because of the recalcitrant nature of the residual hydrocarbon left in the soil.

Furthermore, the number of active wells of the clean soil (soil D) ranged from three to seven wells during the experiments. The AWCD values detected in soil D were in the range 0.1–0.13. They were not significantly different from each other ($P > 0.5$). The number of active wells and AWCD values (0.16–0.19) were relatively low in soil E, indicating no biological activity. Furthermore, the number of active wells in the untreated contaminated soil (soil F) was relatively steady and unchanged during the experiments.

**Table 1**
Mineral nutrient levels, soil texture, and pH in soil samples

| Soil type | Soil texture (% sand/silt/clay) | pH | N (ppm) | P (ppm) | K (ppm) | Mg (ppm) | Organic C (%) |
|---|---|---|---|---|---|---|---|
| A | Sandy loam (75/16/9) | 6.5 | 4.1 | 7 | 90 | 64 | 5.3 |
| B | Sandy loam (64/21/15) | 7.5 | 2.1 | 6 | 130 | 335 | 7 |
| C | Loamy sand (76/21/3) | 8.1 | 4.3 | 4 | 26 | 47 | 2.5 |
| D | Loamy sand (80/15/5) | 7.0 | 5.0 | 8 | 110 | 80 | 0.5 |
| E | Sandy loam (75/10/15) | 6.7 | 4.0 | 10 | 120 | 52 | 4.3 |
| F | Sandy clay loam (65/15/20) | 7.3 | 3.9 | 9 | 150 | 91 | 6.7 |

A, B, C, treated oil-contaminated soil; D, non-contaminated clean soil (control-1); E, non-contaminated agricultural soil (control-2); F, untreated oil-contaminated soil (control-3).

### 3.3. TPH mineralization

We performed preliminary mineralization experiments to find treatment conditions that enhanced hydrocarbon degradation and that could be compared with a soil toxicity study. Soils A–E were provided with a suitable source for the major nutrients (nitrogen, phosphorus, and potassium), moisture, and oxygen.

Fig. 1 shows the time course of hydrocarbon disappearance. Certain similarities and differences are evident in the variously treated soils. The overall maximum decline in TPH between soils A and B was similar; however, it was significantly different for soil C from that in soils A and B ($P < 0.02$). The corresponding hydrocarbon degradation and microbial functional analysis lent prevailing support to the argument that biodegradation was the principal component of the bioremediation process.

The initial soil concentrations of hydrocarbon varied from 13 000 to 15 000 μg $g^{-1}$, 15 000 to 17 000 μg $g^{-1}$, 17 500 to 19 000 μg $g^{-1}$, and 12 000 to 13 900 μg $g^{-1}$ of TPH for soils A, B, C, and F, respectively. Initial hydrocarbon concentrations for soils D and E were not detected. Mineralization of TPH in soil C was 30% of the initial TPH levels after 200 d (Fig. 2); however, hydrocarbon mineralization in soils A and B was in the range of 75–95%, which indicated the presence of viable TPH-degrading microorganisms. In soils A and B, mineralization was observed at a higher rate for the first 50 d; however, mineralization after 50 d was slow (10–20% mineralization was achieved until 200 d). In soil C, there was no significant effect for the first 50 d; however, between day 50 and day 100, the mineralization was enhanced, resulting in a 20% reduction. Furthermore, although adding N, P, and K generally enhanced hydrocarbon degradation in soils A and B, those same additions typically have a small effect, confirming that the biodegradation of soil contaminants may also be affected by other, undefined parameters (Zappi et al., 1996). Soil F's TPH mineralization, on the other hand, was in the range of 10–20%, indicating the presence of stable but lower TPH-degrading microorganisms. The TPH of soil F decreased from 13 000 μg $g^{-1}$ to 11 000 μg $g^{-1}$ after 200 d.

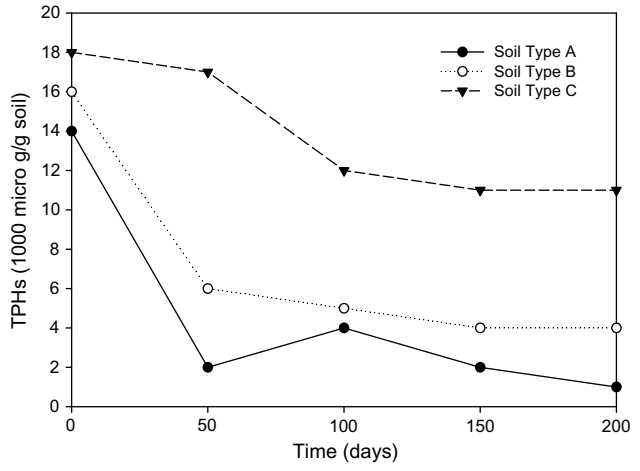


**Fig. 1.** Total petroleum hydrocarbon (TPH) concentrations in soil types A, B, and C during bioremediation in microcosms.

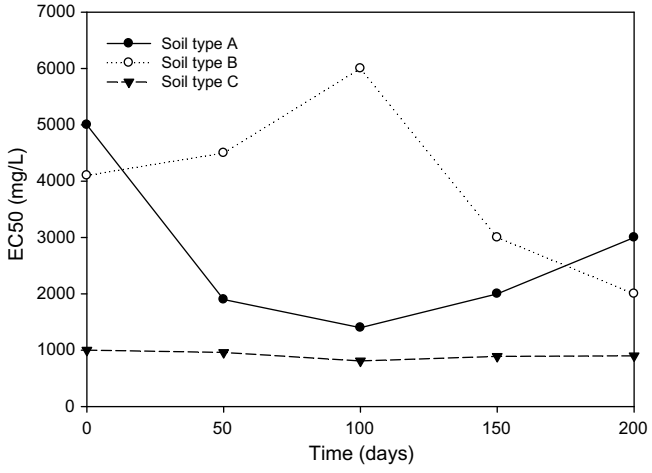


**Fig. 2.** Microtox™ toxicity ($EC_{50}$) of soil types A, B, and C during bioremediation.

### 3.4. Soil toxicity during bioremediation

Solid-phase Microtox $EC_{50}$ values for the three soils are presented in Fig. 2. Soil toxicity was measured before, during, and after bioremediation. The toxicity rating of the samples was performed according to the method of Kwan and Dutka (2004), and the samples were categorized very toxic if the Microtox $EC_{50}$ was $\leqslant$5000 mg $L^{-1}$, moderately toxic at 5000 < $EC_{50} \leqslant$ 10 000 mg $L^{-1}$, and nontoxic at $EC_{50}$ > 10 000 mg $L^{-1}$.

In the undegraded state at time 0, soils A and B exhibited moderate toxicity ($EC_{50}$ = 4000–5000 mg $L^{-1}$) and soil C exhibited very toxic properties ($EC_{50}$ = 1000 mg $L^{-1}$). The subsequent behaviors of the three soils differed in toxicity. The $EC_{50}$ of soil A first decreased (i.e., toxicity increased) from 5000 to 1900 mg $L^{-1}$ after 100 d but started to increase (i.e., toxicity decreased) after 100 d of bioremediation. Curious, however, was the higher toxicity that we found during bioremediation when actually most of the hydrocarbons had disappeared.

Soil B was rapidly detoxified, with $EC_{50}$ values reaching 6000 mg $L^{-1}$ (moderate toxicity) after 100 d of bioremediation (Fig. 2). However, toxicity did increase from days 100 to 200, although there were further reductions in TPHs in soil B. In soil C, $EC_{50}$ values ranged from 850 to 1000 mg $L^{-1}$, with an obvious slight reduction in $EC_{50}$. These apparent increases in toxicity are probably attributable to the formation of intermediary metabolites of hydrocarbon degradation, which are more toxic than their parent compounds. Toxicity of soils D and E, on the other hand, did not appear to change overall for the whole 200 d of bioremediation, and both soils were nontoxic and in the range of 10 000–15 000 mg $L^{-1}$. However, soil F was moderately toxic ($EC_{50}$ from 7000 to 9000 mg $L^{-1}$) for the 200 d of bioremediation.

Furthermore, seed germination ($LC_{50}$) and emergence ($EC_{50}$) data (Figs. 3 and 4) were consistent with the solid-phase toxicity measurements. In soil A, $LC_{50}$ and $EC_{50}$ values decreased from 25% to 9% and increased from 60% to 65% for the first 50 d, respectively. These data suggest that lettuce seed toxicity levels were elevated by decreasing TPH concentrations. Soil A, which was toxic in the seed germination test, could also be considered initially toxic according to the solid-phase Microtox test per criteria suggested by Kwan and Dutka (2004). Seed germination inhibition was stronger for soils A and C, but bioremediation in soil B at least partially restored the ability of the soil to support seed germination and plant growth (Fig. 3).

Furthermore, Microtox and seed germination tests were sensitive to changes in soil toxicity during bioremediation of petroleum hydrocarbon-contaminated soils (Coover and Sims, 1987; Erickson et al., 1993; Benton et al., 1995; Marwood et al., 1998). These data corroborate the Microtox data for early sampling times and suggest the presence of toxic metabolites.

Root elongation toxicity ($EC_{50}$) for soil A increased from 60% to 65% toxicity and then decreased to 50% after 100 d of bioremediation. Compared with soil A toxicity data, it appears that phytotox-

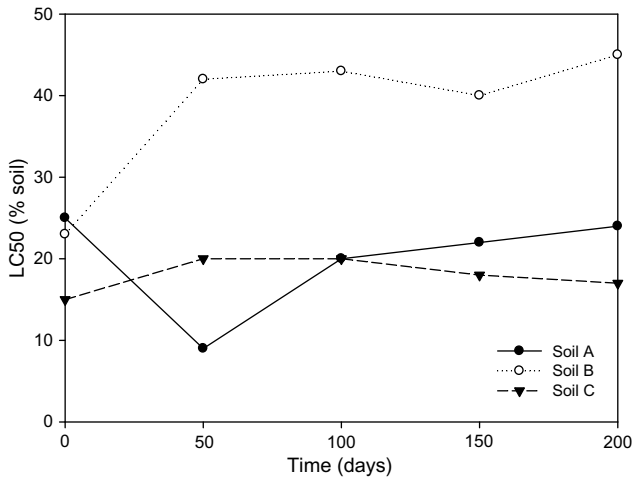


**Fig. 3.** Seed germination $LC_{50}$ values (% of contaminated soil) for the three types of soils during bioremediation in microcosms. Each data point represents the value obtained using probit analysis of total seedlings germinated or emerged on triplicate Petri dishes.

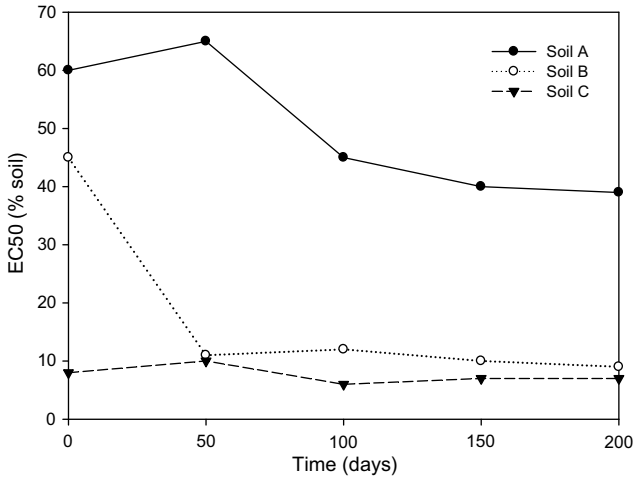


**Fig. 4.** Root elongation $EC_{50}$ values (% of contaminated soil) for the three types of soils during bioremediation in microcosms. Each data point represents the value obtained using probit analysis of total seedlings germinated or emerged on triplicate Petri dishes.

icity of soil B also becomes extremely significant after 50 d of bioremediation. The $EC_{50}$ of soil B decreased from 45% to 10% after 50 d. Also, soil C was also very toxic and the $EC_{50}$ was below 10% toxicity.

Soils D and E were both nontoxic ($LC_{50}$ and $EC_{50}$) and in the range of 60–70% throughout the bioremediation process. On the other hand, soil F showed moderate toxicity for seed germination ($LC_{50}$ = 60–75%) and for emergence ($EC_{50}$ = 70–80%) throughout the bioremediation process.

The parallel measurements of solid-phase Microtox, seed germination, and emergence in soils mutually support each other in describing the fate and effects of a particular crude oil spill. The two assays agreed in suggesting that toxicity had initially increased in soil A. The toxicity test data obtained in this study did not indicate that bioremediation had successfully reduced soil toxicity, although the treatment conditions were chosen based on successful mineralization of TPH in previous experiments. This finding suggests that the bioavailability, degradation, and toxicity of soil contaminants are all influenced by sorption, which is influenced by time and the physicochemical properties of individual soils (Dorn et al., 1998; Trevors, 1999; Cai et al., 2007). Although there are advantages to testing solid soil, each soil is unique in the response that treatment induces, and each toxicity test is unique in its ability to detect different contaminant levels in different soils. Therefore, it appears that, although soil toxicity tests can be used to monitor bioremediation, chemical and toxicity data do not always corroborate one another.

### 3.5. Leaching potential of the contaminated soil

We applied the standard TCLP approach to all soil samples. Metal concentrations in the TCLP leachates for 0 and 200 d of bioremediation time for soils A, B, and C are given in Table 2. The three bioremediated soils had a different effect on leaching. The metals leached in detectable concentrations at day 0 for all samples were As, Cd, Cr, and Pb; however, at day 200, only soil A did not leach any metals above the TCLP requirements. Soil A's leaching potential of metals was generally low, lower than TCLP requirements. The metals were reduced in the leachates because of the fixation capability of this type of soil medium after 200 d of bioremediation, thereby lowering the bioavailability and reducing the mobility of these metals. The highest leaching metal for soil B was Cd at day 200, reaching up to 9 mg $L^{-1}$, whereas the highest leaching metals for soil C were Cd, Cr, and Pb, reaching up to 21, 7, and 41 mg $L^{-1}$, respectively.

On average, Cd and Pb concentrations in the leachates of soil C were, respectively, 20-fold higher than in the leachates of soil A (Cd, $P < 0.001$; Pb, $P < 0.002$). Soil C almost doubled the leachability of Cr compared with soil A ($P < 0.001$). The results showed that the level of Ba leaching from soil B was 39 mg $L^{-1}$, which was higher than the leachate concentration in both soils A and B. The increased metal leaching was ascribed to the formation of metal–organic complexes or chelating compounds inside the soil, but changes in chemical properties of soil could also have an effect. Leaching potentials of soils D and E were generally negligible and were below the detection level throughout bioremediation. Furthermore, soil F leached detectable concentrations of Cd and Pb at day 0 and day 200 (Table 2).

Also, it has been recognized that the predominant leachable components from petroleum-containing wastes are the more water-soluble hydrocarbons (benzene, toluene, ethylbenzene, and xylenes [BTEX]). Table 3 shows residual BTEX components for soils A, B, C, and F at day 0 and day 200 of bioremediation. BTEX was detected at high levels at day 0 in soils C and F. After 200 d of bioremediation, soils A, B, and C contained little or no detectable benzene levels in the leachates. However, soil F benzene levels were higher than 19 mg $kg^{-1}$. Also, the TEX concentration was reduced in soil C from 256 to 1.9 mg $kg^{-1}$ during the same period.

**Table 2**
Metal concentration in the TCLP leachates for 0 and 200 d of bioremediation

| Soil type | Bioremediation time (d) | Metal concentration (mg $L^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | As | Ba | Cd | Cr | Pb | Se |
| A | 0 | 9.2 | 48 | 31 | 6.6 | 2500 | 7 |
| | 200 | ND | 12 | ND | 1 | 2 | ND |
| B | 0 | 5 | 79 | 76 | 11 | 1290 | 3 |
| | 200 | 1 | 39 | 9 | 3 | 4 | ND |
| C | 0 | 120 | 62 | 71 | 18 | 3600 | 2 |
| | 200 | 1 | 2 | 21 | 2 | 41 | ND |
| D | 0 | 1 | 3 | 95 | 2 | 1600 | 5 |
| | 200 | ND | ND | 82 | ND | 1200 | ND |

The leaching test (TCLP) requirements are <5 for As, <100 for Ba, <1 for Cd, <5 for Cr, <5 for Pb, and <1 for Se.

**Table 3**
BTEX leaching potential of the TCLP leachate method for soil types A, B, and C at 0 and 200 d of bioremediation

| Soil type | Bioremediation time (d) | Benzene (B) (mg $kg^{-1}$) | Toluene, ethylbenzene, and xylenes (TEX) (mg $kg^{-1}$) |
|---|---|---|---|
| A | 0 | 2.1 | 2.1 |
| | 200 | ND | ND |
| B | 0 | 2.9 | 41 |
| | 200 | ND | ND |
| C | 0 | 63.1 | 256 |
| | 200 | 3.1 | 1.9 |
| D | 0 | 29 | 160 |
| | 200 | 19 | 120 |

Although residual TEX from soils A and B at day 0 (2.1 and 41 mg $kg^{-1}$) were detected, these residuals were below the detection levels after 200 d of bioremediation. TEX levels of soil F decreased from 160 to 120 mg $kg^{-1}$. Furthermore, the residual BTEX levels for soils D and E were not detected throughout the bioremediation process.

Moreover, the toxicological properties of the leachates produced by the TCLP were assessed by using *V. fischeri* as a test organism. Soil leachate samples exhibited extreme to moderate toxicity to *V. fischeri*, which sometimes reached 800–1000 mg $L^{-1}$ bioluminescence level of toxicity in soils C and D after 200 d of bioremediation. Similar to TCLP toxicity results, toxicity of soil B (6000 mg $L^{-1}$) presented lower values than that of soil A (4000 mg $L^{-1}$). The toxicity decreased in soil B because of the corresponding decrease in leached metal concentrations.

In general, the beneficial role of bioremediation on the leachability of toxic components could be attributed to the formation of stable structures, which may enhance the stabilization of metals and reduce their release; at prolonged times, the decomposition of other chemicals may contribute to the increased release of toxic components. Thus, the toxic compounds binding to the contaminated soil will reduce both their leach-ability to groundwater and absorption by plants. Also, the high toxic effects of the eluates to *V. fischeri* obtained by the leaching procedure may be attributed to the increased sensitivity of the particular species to high metal concentrations.

## 4. Conclusion

The combination of bioassays used in this study for examination of petroleum hydrocarbon-contaminated soil after bioremediation was not comparable to data from chemical testing. The findings that contaminants were significantly reduced did not match the toxicity findings. There is absolutely a need to develop environmentally acceptable toxicological endpoints for soil quality and an integrated approach to estimate ecological risk. The Microtox system and seed germination tests could be used to assess the efficacy of response to mitigative actions, chemical cleaning, mechanical removal, and "no treatment" options. The results provide additional information to environmental engineers who must decide whether additional cleanup, such as bioremediation, is needed. Both toxicological tests were simple and economical to conduct and have good potential application as environmental monitoring tools to assess the efficacy of a remediation technology for site cleanup. Finally, water-soluble hydrocarbons (BTEX) could be leached from pretreated soils. However, after 200 d of bioremediation, BTEX components were no longer leachable from the three soils. These data demonstrate that treated oily soils lose their potential to leach significant amounts of BTEX.

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